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WO 2004/052357 A1

(54) Title: LABELED MACROPHAGE SCAVENGER RECEPTOR ANTAGONISTS FOR IMAGING CARDIOVASCULAR DISEASES

(57) Abstract: The present invention is in the field of diagnostic imaging. In one aspect, the invention relates to novel imaging agents comprising synthetic macrophage scavenger receptor A antagonists, said imaging agents being useful in the diagnostic imaging of cardiovascular disease. Also claimed in the present invention is a pharmaceutical composition comprising the novel imaging agents of the invention, said pharmaceutical composition being useful for the diagnostic imaging of cardiovascular disease in humans. Another aspect of the present invention is a kit useful in the preparation of the pharmaceutical composition of the invention. Furthermore, the use of the imaging agent of the invention for the diagnostic imaging of cardiovascular disease is also claimed.

LABELED MACROPHAGE SCAVENGER RECEPTOR ANTAGONISTS FOR IMAGING CARDIOVASCULAR DISEASES

Technical Field of the Invention

The present invention relates to the field of *in vivo* diagnostic imaging. In particular the present invention relates to novel imaging agents comprising macrophage scavenger receptor antagonists, said novel imaging agents being useful in *in vivo* diagnostic imaging.

Background and Description of Related Art

Cardiovascular disease (CVD) is the leading cause of death in the Western world and encompasses dysfunctional conditions of the heart, arteries, veins and lungs that supply oxygen to vital life-sustaining areas of the body like the brain, the heart itself, and other vital organs. These conditions include coronary heart disease (CHD), coronary artery disease (CAD), chronic obstructive pulmonary disease (COPD), atherosclerosis, and thrombosis, and can lead to potentially life-threatening events as myocardial infarction (MI), pulmonary embolism (PE) and stroke. One factor in common to all these conditions is the involvement of macrophages.

CHD is the most prevalent of the cardiovascular diseases. In 1998 it is estimated that CHD was the cause of 7 million deaths worldwide. CAD precedes CHD, and in the majority of cases the underlying cause is atherosclerosis. Atherosclerosis is a benign disease for many decades until the atherosclerotic plaque becomes atheromatous and potentially symptom producing. The plaque can obstruct blood flow resulting in stenosis of the artery, leading to acute myocardial ischemia in the case of coronary arteries. Additionally, mature atherosclerotic plaques can rupture resulting in the release of thrombogenic lipid, and this plaque component can form a thrombosis that completely blocks the artery. Angina is a common manifestation of CHD and is often the forerunner to more serious complications such as acute coronary syndromes including unstable angina, myocardial infarction and sudden cardiac death. Plaque rupture

precedes the majority of clinical events and the vulnerability of plaques is the most important predictor of clinical outcome.

Macrophage scavenger receptors (MSRs) are expressed on resident macrophages in tissues such as lung, liver, spleen, and recognise modified forms of low-density lipoprotein (LDL). They are not expressed on circulating cells. Class A MSR (MSRA) is known to have a role in the development of atherosclerotic plaques, MSRA I and MSRA II being responsible for the uptake of oxidised LDL and acetylated LDL into macrophages. MSRA expression is an indicator of the lipid burden of macrophages, and therefore may indicate instability of an atherosclerotic plaque.

A series of MSRA antagonists have been reported as being useful in the treatment of CVD. These include salicylanilide derivatives (WO 99/07382), isophthalic acid derivatives (WO 00/06147), phenylenediamines (WO 00/03704) and sulfonamidobenzanilide derivatives (WO 00/78145 and WO 01/98264). The cited documents disclose pharmaceutical compositions comprising these compounds for the treatment of CVD in humans. In addition to being useful in the treatment of CVD, the cited documents also disclose that these compounds may be used in methods for antagonising the MSRA in animals as well as methods for inhibiting lipid accumulation within macrophage-derived foam cells.

WO 02/067761 discloses detectably labelled MSRA antagonists as being useful in the diagnosis and monitoring of CVD. The MSRA antagonists of WO 02/067761 are salicylanilide derivatives, isophthalic acid derivatives and phenylenediamine derivatives. MSRA antagonists that are sulphonamidobenzamide compounds are not disclosed. The IC_{50} values for the compounds of WO 02/067761 are disclosed as <100mM in binding/uptake assays. No specific examples of particular compounds tested are given in that document. The compounds of the present invention have been shown to display superior binding characteristics in comparison to those reported in WO 02/067761.

Summary of the Present Invention

Novel imaging agents comprising synthetic MSRA antagonists have now been identified that possess superior properties over the prior art compounds for diagnosis and monitoring of CVD as well as neurological conditions in which microglia are involved.

An MSRA antagonist is attached to an imaging moiety, said imaging moiety being suitable for the *in vivo* detection of the MSRA antagonist using known diagnostic imaging modalities. Suitable synthetic MSRA antagonists of the present invention are sulphonamidobenzamide compounds. The imaging agents of the invention display superior properties for imaging compared with the prior art compounds.

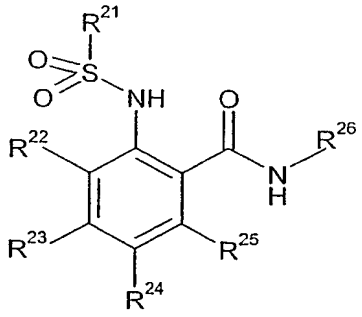
Also disclosed in the present invention is a pharmaceutical composition comprising the novel imaging agent of the present invention and kits for the preparation of said pharmaceutical composition. Furthermore, the present invention discloses a method of imaging CVD using the novel imaging agent of the invention.

Detailed Description of the Invention

The compounds of the invention are useful for diagnostic imaging of CVD. "CVD" as defined in the present invention includes such disease states as atherosclerosis, CAD, thrombosis, transient ischaemia and renal disease. The compounds of the invention are also useful for diagnostic imaging of neurological diseases where monocyte-derived nervous system cells called microglia are implicated such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and encephalitis.

A first aspect of the invention is an imaging agent which comprises a synthetic MSRA antagonist labelled with an imaging moiety, wherein the synthetic MSRA antagonist is a sulphonamidobenzamide compound, and wherein the imaging moiety can be detected externally in a non-invasive manner following administration of said labelled synthetic MSRA antagonist to the mammalian body *in vivo*.

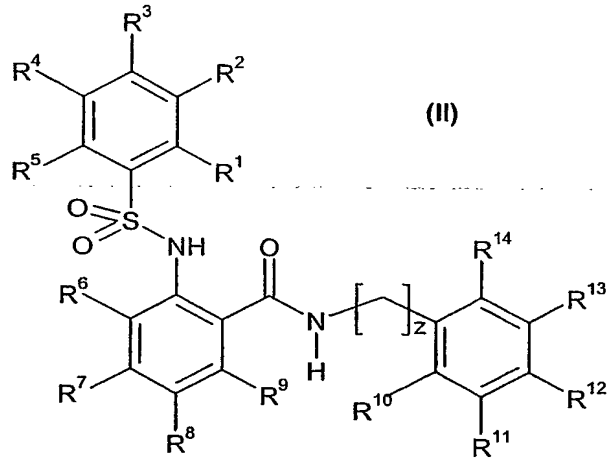
Suitable sulphonamidobenzamide compounds of the invention are of Formula (I):



(I)

wherein R^{21} to R^{26} are independently selected from hydrogen, C_{1-6} alkyl, C_{6-14} aryl, carboxy, amino, hydroxy, or methoxy and wherein one or more of R^{22} to R^{25} may alternatively be a halogen.

A preferred sulphonamidobenzamide compound of the invention is of Formula (II):



(II)

wherein;

z is 0, 1 or 2;

R^1-R^{14} are independently R groups, where R is;

hydrogen, hydroxy, carboxy, C_{1-6} alkyl, nitro, cyano, amino, halogen, C_{6-14} aryl, alkenyl, alkynyl, acyl, aroyl, carboalkoxy, carbamoyl, carbamyl, alkysulphinyl, arylsulphinyl,

arylalkylsulphinyl, alkylsulphonyl, arylsulphonyl, arylalkylsulphonyl, sulphamyl, arylsulphonamido or alkylsulphonamido.

A preferred imaging agent of the invention is of Formula (II) wherein each R^1 to R^{14} is chosen from an imaging moiety, hydrogen, C_{1-6} alkyl, hydroxy, carboxy, amino or halogen.

A most preferred imaging agent of the invention is of Formula (II) wherein one of R^2, R^3, R^7, R^8 and R^{12} is an imaging moiety, and the remaining R^2, R^3, R^7, R^8 and R^{12} groups are independently selected from hydrogen, C_{1-6} alkyl, carboxy, or a halogen selected from chlorine, bromine, fluorine or iodine.

An especially preferred imaging agent of the invention is of Formula (II) wherein R^3, R^8 and R^{12} are all halogens with at least one being an imaging moiety.

The sulphonamidobenzamide compounds of the invention can be prepared as described in Scheme 1 of WO 00/78145. A synthesis of a compound of Formula (II) where z = 0 is illustrated in Figure 1. R^1 to R^{14} are as defined for Formula (II) above. Similar syntheses may be used for the preparation of compounds of Formula (II) wherein z = 1 and z = 2.

"Alkyl" used either alone or as part of another group is defined herein as any straight, branched or cyclic, saturated or unsaturated C_nH_{2n+1} group, wherein unless otherwise specified n is an integer between 1 and 6. The term alkyl in the present invention is also taken to include substituted alkyls, e.g. hydroxyalkyls, haloalkyls, aminoalkyls, carboxyalkyls and alkoxyalkyls.

"Aryl" used either alone or as part of another group is defined herein as any C_{6-14} molecular fragment or group which is derived from a monocyclic or polycyclic aromatic hydrocarbon. Suitable aryl groups of the invention include, but are not limited to, haloaryl, alkylaryl, arylcarbamyl, phenylazo, arylamino, arylthio, toluene, benzoic acid,

phenol, arylsulfinyl, arylsulfonyl, arylsulfonamido, benzothiophene, naphthalene, quinoline, isoquinoline, pyridine, pyrimidine, and pyrazine.

The term "halogen" means a group selected from fluorine, chlorine, bromine, and iodine or isotopes thereof.

The "imaging moiety" is preferably chosen from:

(i) a radioactive metal ion;
(ii) a paramagnetic metal ion;
(iii) a gamma-emitting radioactive halogen;
(iv) a positron-emitting radioactive non-metal;
(v) a hyperpolarised NMR-active nucleus;
(vi) a reporter suitable for *in vivo* optical imaging;
(vii) a β-emitter suitable for intravascular detection.

When the imaging moiety is a radioactive metal ion, i.e. a radiometal, suitable radiometals can be either positron emitters such as ^{64}Cu, ^{48}V, ^{52}Fe, ^{55}Co, ^{94m}Tc or ^{68}Ga; γ-emitters such as ^{99m}Tc, ^{111}In, ^{113m}In, or ^{67}Ga. Preferred radiometals are ^{99m}Tc, ^{64}Cu, ^{68}Ga and ^{111}In. Most preferred radiometals are γ-emitters, especially ^{99m}Tc.

When the imaging moiety is a paramagnetic metal ion, suitable such metal ions include: Gd(III), Mn(II), Cu(II), Cr(III), Fe(III), Co(II), Er(II), Ni(II), Eu(III) or Dy(III). Preferred paramagnetic metal ions are Gd(III), Mn(II) and Fe(III), with Gd(III) being especially preferred.

When the imaging moiety is a gamma-emitting radioactive halogen, the radiohalogen is suitably chosen from ^{123}I, ^{125}I, ^{131}I or ^{77}Br. A preferred gamma-emitting radioactive halogen is ^{123}I.

When the imaging moiety is a positron-emitting radioactive non-metal, suitable such positron emitters include: ^{11}C, ^{13}N, ^{15}O, ^{17}F, ^{18}F, ^{75}Br, ^{76}Br or ^{124}I. Preferred positron-

emitting radioactive non-metals are ^{11}C, ^{13}N and ^{18}F, especially ^{11}C and ^{18}F, most especially ^{18}F.

When the imaging moiety is a hyperpolarised NMR-active nucleus, such NMR-active nuclei have a non-zero nuclear spin, and include ^{13}C, ^{15}N, ^{19}F, ^{29}Si and ^{31}P. Of these, ^{13}C is preferred. By the term "hyperpolarised" is meant enhancement of the degree of polarisation of the NMR-active nucleus over its' equilibrium polarisation. The natural abundance of ^{13}C (relative to ^{12}C) is about 1%, and suitable ^{13}C-labelled compounds are suitably enriched to an abundance of at least 5%, preferably at least 50%, most preferably at least 90% before being hyperpolarised. At least one carbon atom of the sulphonamidobenzamide compound of the present invention is suitably enriched with ^{13}C, which is subsequently hyperpolarised.

When the imaging moiety is a reporter suitable for *in vivo* optical imaging, the reporter is any moiety capable of detection either directly or indirectly in an optical imaging procedure. The reporter might be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter. More preferably the reporter is a dye such as a chromophore or a fluorescent compound. The dye can be any dye that interacts with light in the electromagnetic spectrum with wavelengths from the ultraviolet light to the near infrared. Most preferably the reporter has fluorescent properties.

Preferred organic chromophoric and fluorophoric reporters include groups having an extensive delocalized electron system, e.g. cyanines, merocyanines, indocyanines, phthalocyanines, naphthalocyanines, triphenylmethines, porphyrins, pyrilium dyes, thiapyriliup dyes, squarylium dyes, croconium dyes, azulenium dyes, indoanilines, benzophenoxazinium dyes, benzothiaphenothiazinium dyes, anthraquinones, napthoquinones, indathrenes, phthaloylacridones, trisphenoquinones, azo dyes, intramolecular and intermolecular charge-transfer dyes and dye complexes, tropones, tetrazines, *bis*(dithiolene) complexes, *bis*(benzene-dithiolate) complexes, iodoaniline dyes, *bis*(S,O-dithiolene) complexes. Fluorescent proteins, such as green fluorescent protein (GFP) and modifications of GFP that have different absorption/emission properties are also useful. Complexes of certain rare earth metals (e.g., europium,

samarium, terbium or dysprosium) are used in certain contexts, as are fluorescent nanocrystals (quantum dots).

Particular examples of chromophores which may be used include: fluorescein, sulforhodamine 101 (Texas Red), rhodamine B, rhodamine 6G, rhodamine 19, indocyanine green, Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, Marina Blue, Pacific Blue, Oregon Green 88, Oregon Green 514, tetramethylrhodamine, and Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 532, Alexa Fluor 546, Alexa Fluor 555, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 647, Alexa Fluor 660, Alexa Fluor 680, Alexa Fluor 700, and Alexa Fluor 750.

Particularly preferred are dyes which have absorption maxima in the visible or near infrared region, between 400 nm and 3 μm, particularly between 600 and 1300 nm.

Optical imaging modalities and measurement techniques include, but not limited to: luminescence imaging; endoscopy; fluorescence endoscopy; optical coherence tomography; transmittance imaging; time resolved transmittance imaging; confocal imaging; nonlinear microscopy; photoacoustic imaging; acousto-optical imaging; spectroscopy; reflectance spectroscopy; interferometry; coherence interferometry; diffuse optical tomography and fluorescence mediated diffuse optical tomography (continuous wave, time domain and frequency domain systems), and measurement of light scattering, absorption, polarisation, luminescence, fluorescence lifetime, quantum yield, and quenching.

When the imaging moiety is a β-emitter suitable for intravascular detection, suitable such β-emitters include the radiometals ^{67}Cu, ^{89}Sr, ^{90}Y, ^{153}Sm, ^{186}Re, ^{188}Re or ^{192}Ir, and the non-metals ^{32}P, ^{33}P, ^{38}S, ^{38}Cl, ^{39}Cl, ^{82}Br and ^{83}Br.

Whichever imaging moiety is selected from the above, it is preferably reacted with a precursor of said imaging agent. An imaging agent precursor constitutes a second aspect of the present invention. Reaction of such a precursor with a suitable chemical form of the imaging moiety results in the production of said imaging agent. A "precursor"

as defined in the present invention is a MSRA antagonist compound to which an imaging moiety may be readily attached, preferably in a one-step process. One example of a suitable precursor of the invention is a MSRA antagonist conjugated to a metal chelating agent, suitable for the attachment of an imaging moiety which is a metal ion. Another example of a suitable precursor of the invention is a MSRA antagonist that includes a group such as (a) a non-radioactive halogen atom, (b) an activated aryl ring, (c) an organometallic precursor compound, or (d) an organic precursor such as triazene. Such a precursor is suitable for the incorporation of an imaging moiety which is a radioactive halogen. These precursor compounds and the resultant imaging agents are described more fully in the following sections.

When the imaging moiety comprises a metal ion, the metal ion is suitably attached to the MSRA antagonist as part of a conjugated metal complex of Formula (III):

[{MSRA antagonist}-$(L)_x]_y$-[metal complex] (III)

wherein:

$-(L)_x-$ is a linker group wherein each L is independently $-CZ_2-$, $-CZ=CZ-$, $-C\equiv C-$, $-CZ_2CO_2-$, $-CO_2CZ_2-$, $-NZCO-$, $-CONZ-$, $-NZ(C=O)NZ-$, $-NZ(C=S)NZ-$, $-SO_2NZ-$, $-NZSO_2-$, $-CZ_2OCZ_2-$, $-CZ_2SCZ_2-$, $-CZ_2NZCZ_2-$, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, a C_{3-12} heteroarylene group, an amino acid or a monodisperse polyethyleneglycol (PEG) building block;

Z is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

x is an integer of value 0 to 10; and

y is 1, 2 or 3.

By the term "metal complex" is meant a coordination complex of the metal ion with one or more ligands. It is strongly preferred that the metal complex is "resistant to transchelation", i.e. does not readily undergo ligand exchange with other potentially competing ligands for the metal coordination sites. Potentially competing ligands include the synthetic MSRA antagonist itself plus other excipients in the preparation *in vitro* (e.g. radioprotectants or antimicrobial preservatives used in the preparation), or endogenous compounds *in vivo* (e.g. glutathione, transferrin or plasma proteins). The "linker group" $(L)_x$ is as defined below for Formula (IIIa).

The metal complexes of Formula (III) are conveniently prepared from precursors which are ligand conjugates of Formula (IIIa):

[{MSRA antagonist}-$(L)_x$]$_y$-[ligand] **(IIIa)**

wherein $(L)_x$, x and y are as defined for Formula (III) above.

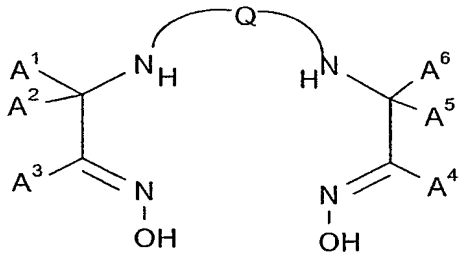
In Formulae (III) and (IIIa), y is preferably 1 or 2, and is most preferably 1.

Suitable ligands for use in the present invention, which form metal complexes resistant to transchelation, include chelating agents which have 2-6, preferably 2-4, metal donor atoms arranged such that 5- or 6-membered chelate rings result (by having a non-coordinating backbone of either carbon atoms or non-coordinating heteroatoms linking the metal donor atoms). Examples of donor atom types which bind well to metals as part of chelating agents are: amines, thiols, amides, oximes and phosphines. Phosphines form such strong metal complexes that even monodentate or bidentate phosphines form suitable metal complexes. The linear geometry of isonitriles and diazenides is such that they do not lend themselves readily to incorporation into chelating agents, and are hence typically used as monodentate ligands. Examples of suitable isonitriles include simple alkyl isonitriles such as *tert*-butylisonitrile, and ether-substituted isonitriles such as MIBI (i.e. 1-isocyano-2-methoxy-2-methylpropane). Examples of suitable phosphines include Tetrofosmin, and monodentate phosphines

such as *tris*(3-methoxypropyl)phosphine. Examples of suitable diazenides include the HYNIC series of ligands, i.e. hydrazine-substituted pyridines or nicotinamides.

Examples of suitable chelating agents for technetium which form metal complexes resistant to transchelation include, but are not limited to:

(i) diaminedioximes of Formula (IV):



(IV)

where A^1-A^6 are each independently an A group;

each A is H or C_{1-10} alkyl, C_{3-10} alkylaryl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} fluoroalkyl, C_{2-10} carboxyalkyl or C_{1-10} aminoalkyl, or two or more A groups together with the atoms to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring, and wherein one or more of the A groups is conjugated to the MSRA antagonist;

and Q is a bridging group of Formula $-(J)_m-$;

where m is 3, 4 or 5 and each J is independently -O-, -NA- or $-C(A)_2-$ provided that $-(J)_m-$ contains a maximum of one J group which is -O- or -NA-.

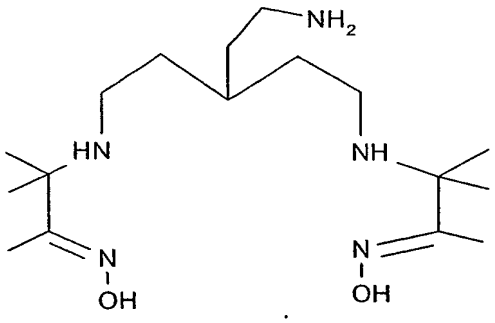
Preferred Q groups are as follows:

$Q = -(CH_2)(CHA)(CH_2)-$ i.e. propyleneamine oxime or PnAO derivatives;
$Q = -(CH_2)_2(CHA)(CH_2)_2-$ i.e. pentyleneamine oxime or PentAO derivatives;
$Q = -(CH_2)_2NA(CH_2)_2-$.

A^1 to A^6 are preferably chosen from: C_{1-3} alkyl, alkylaryl alkoxyalkyl, hydroxyalkyl, fluoroalkyl, carboxyalkyl or aminoalkyl. Most preferably, each A^1 to A^6 group is CH_3.

The synthetic MSRA antagonist is preferably conjugated at either A^1 or A^6, or an A group of the Q moiety. Most preferably, the MSRA antagonist is conjugated to an A group of the Q moiety. When the MSRA antagonist is conjugated to an A group of the Q moiety, the A group is preferably at the bridgehead position. In that case, Q is preferably $-(CH_2)(CHA)(CH_2)-$, $-(CH_2)_2(CHA)(CH_2)_2-$ or $-(CH_2)_2(NA)(CH_2)_2-$, most preferably $-(CH_2)_2(CHA)(CH_2)_2-$.

An especially preferred bifunctional diaminedioxime chelator is of Formula (V):



(V)

This chelator will also be referred to as "chelating agent 1". The synthetic MSRA antagonist is conjugated to chelating agent 1 *via* the bridgehead NH_2 group.

(ii) N_3S ligands having a thioltriamide donor set such as MAG_3 and related ligands; or having a diamidepyridinethiol donor set such as PICA;

(iii) N_2S_2 ligands having a diaminedithiol donor set such as BAT or ECD (i.e. ethylcysteinate dimer), or an amideaminedithiol donor set such as MAMA;

(iv) N_4 ligands which ore open chain or macrocyclic ligands having a tetramine, amidetriamine or diamidediamine donor set, such as cyclam, monoxocyclam or dioxocyclam; and,

(v) N_2O_2 ligands having a diaminediphenol donor set.

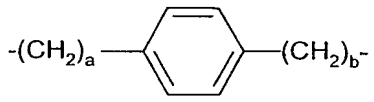
The above described ligands are particularly suitable for complexing technetium, e.g. ^{94m}Tc or ^{99m}Tc, and are described more fully by Jurisson *et al* [Chem.Rev., 99, 2205-2218 (1999)]. The ligands are also useful for other metals, such as copper (^{64}Cu or ^{67}Cu), vanadium (e.g. ^{48}V), iron (e.g. ^{52}Fe), or cobalt (e.g. ^{55}Co). Other suitable ligands are described in Sandoz WO 91/01144, which includes ligands which are particularly suitable for indium, yttrium and gadolinium, especially macrocyclic aminocarboxylate and aminophosphonic acid ligands. Ligands which form non-ionic (i.e. neutral) metal complexes of gadolinium are known and are described in US 4885363. When the radiometal ion is technetium, the ligand is preferably a chelating agent which is tetradentate. Preferred chelating agents for technetium are the diaminedioximes, or those having an N_2S_2 or N_3S donor set as described above. Especially preferred chelating agents for technetium are the diaminedioximes.

It is envisaged that the role of the linker group $-(L)_x-$ in Formula (III) and (IIIa) is to distance the relatively bulky metal complex which results upon metal co-ordination, from the active site of the MSRA antagonist, so that binding of the antagonist to MSRA is not impaired. This can be achieved by a combination of flexibility (e.g. simple alkyl chains), so that the bulky group has the freedom to position itself away from the active site and/or rigidity such as a cycloalkyl or aryl spacer which orientates the metal complex away from the active site.

The nature of the linker group can also be used to modify the biodistribution of the resulting metal complex of the conjugate. Thus, e.g. the introduction of ether groups in the linker will help to minimise plasma protein binding. Preferred linker groups have a backbone chain of linked atoms which make up the $(L)_x$ moiety containing 2 to 10 atoms, most preferably 2 to 5 atoms, with 2 or 3 atoms being especially preferred. A minimum linker group backbone chain of 2 atoms confers the advantage that the ligand is well-separated from the MSRA antagonist so that any interaction is minimised.

Non-peptide linker groups such as alkylene groups or arylene groups have the advantage that there are no significant hydrogen bonding interactions with the

conjugated MSRA antagonist, so that the linker does not wrap round onto the MSRA antagonist. Preferred alkylene spacer groups are $(CH_2)_q$ where q is 2 to 5. Preferred arylene spacers are of Formula (VI):



(VI)

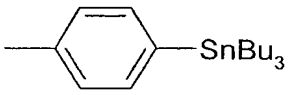
where: a and b are independently 0, 1 or 2.

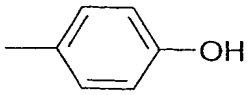
It is strongly preferred that the metal complex is bound in such a way that the linkage does not undergo facile metabolism in blood, since that would result in the metal complex being cleaved off before the imaging agent reaches the desired *in vivo* target site. The metal complexes are preferably covalently bound *via* linkages which are not readily metabolised.

When the imaging moiety is a radioactive halogen, it is preferably a radioactive isotope of iodine. The radioiodine atom is preferably attached *via* a direct covalent bond to an aromatic ring such as a benzene ring, or a vinyl group since it is known that iodine atoms bound to saturated aliphatic systems are prone to *in vivo* metabolism and hence loss of the imaging moiety.

When the imaging moiety is radioactive halogen, such as iodine, suitable precursors of the imaging agent include: a non-radioactive halogen atom such as an aryl iodide or aryl bromide (to permit radioiodine exchange); an activated aryl ring (e.g. a phenol group); or an organometallic precursor compound (e.g. trialkyltin or trialkylsilyl), an organic precursor such as triazenes or other such moiety known to those skilled in the art. Methods of introducing radioactive halogens (including ^{123}I and ^{18}F) are described by Bolton [J.Lab.Comp.Radiopharm., 45, 485-528 (2002)].

Examples of suitable aryl groups to which radioactive halogens, especially iodine can be attached are given below:





Both contain substituents which permit facile radioiodine substitution onto the aromatic ring. Alternative substituents containing radioactive iodine can be synthesised by direct iodination, e.g. *via* radiohalogen exchange:

When the imaging moiety comprises a radioactive isotope of fluorine (eg. ^{18}F), the radioiodine atom may be carried out *via* direct labelling using the reaction of ^{18}F-fluoride with a suitable precursor having a good leaving group, such as an alkyl bromide, alkyl mesylate or alkyl tosylate. ^{18}F can also be introduced by N-alkylation of amine precursors with alkylating agents such as $^{18}F(CH_2)_3OMs$ (where Ms is mesylate) to give N-$(CH_2)_3{}^{18}F$, or O-alkylation of hydroxyl groups with $^{18}F(CH_2)_3OMs$ or $^{18}F(CH_2)_3Br$. For aryl systems, ^{18}F-fluoride displacement of nitrogen from an aryl diazonium salt is a possible route to aryl-^{18}F derivatives. See Bolton, J.Lab.Comp.Radiopharm., 45, 485-528 (2002) for a description of routes to ^{18}F-labelled derivatives.

In a third aspect of the invention a pharmaceutical composition comprising the imaging agent of the invention together with a biocompatible carrier, in a form suitable for mammalian administration, is disclosed.

A "pharmaceutical composition" is defined in the present invention as a formulation comprising the imaging agent of the invention or a salt thereof in a form suitable for administration to humans. The pharmaceutical composition of the invention is preferably administered parenterally, i.e. by injection, and most preferably as an aqueous solution. Such a formulation may optionally contain further ingredients such as buffers; pharmaceutically acceptable solubilisers (e.g. cyclodextrins or surfactants such

as Pluronic, Tween or phospholipids); pharmaceutically acceptable stabilisers or antioxidants (such as ascorbic acid, gentisic acid or *para*-aminobenzoic acid).

In a fourth aspect of the invention, a kit for the preparation of the pharmaceutical composition of the invention is disclosed which comprises a precursor of the imaging agent of the invention. Such kits are designed to give sterile products suitable for human administration, e.g. *via* direct injection into the bloodstream, and comprise a precursor of said imaging agent.

Preferably, the kit is for the preparation of a pharmaceutical composition which comprises an imaging agent wherein the imaging moiety is selected from a radioactive metal ion, a paramagnetic metal ion, or a radiohalogen. The precursor in each case is as described earlier in the description, e.g. Formula (IIIa) for metal ions.

Where the radiometal is ^{99m}Tc, the kit is preferably lyophilised and is designed to be reconstituted with sterile ^{99m}Tc-pertechnetate (TcO_4^-) from a ^{99m}Tc radioisotope generator to give a solution suitable for human administration without further manipulation. Suitable kits comprise a container (e.g. a septum-sealed vial) containing the MSRA antagonist-chelating agent conjugate in either free base or acid salt form, together with a pharmaceutically acceptable reducing agent such as sodium dithionite, sodium bisulphite, ascorbic acid, formamidine sulphinic acid, stannous ion, Fe(II) or Cu(I). The pharmaceutically acceptable reducing agent is preferably a stannous salt such as stannous chloride or stannous tartrate. Alternatively, the kit may optionally contain a metal complex, which upon addition of the radiometal, undergoes transmetallation (i.e. metal exchange) giving the desired product.

Kits for the preparation of the imaging agents of the invention may optionally further comprise additional components such as a transchelator, radioprotectant, antimicrobial preservative, pH-adjusting agent or filler. The "transchelator" is a compound which reacts rapidly to form a weak complex with technetium, then is displaced by the diaminedioxime. This minimises the risk of formation of reduced hydrolysed technetium (RHT) due to rapid reduction of pertechnetate competing with technetium complexation.

Suitable such transchelators are salts of a weak organic acid, i.e. an organic acid having a pKa in the range 3 to 7, with a biocompatible cation. Suitable such weak organic acids are acetic acid, citric acid, tartaric acid, gluconic acid, glucoheptonic acid, benzoic acid, phenols or phosphonic acids. Hence, suitable salts are acetates, citrates, tartrates, gluconates, glucoheptonates, benzoates, phenolates or phosphonates. Preferred such salts are tartrates, gluconates, glucoheptonates, benzoates, or phosphonates, most preferably phosphonates, most especially diphosphonates. A preferred such transchelator is a salt of MDP, i.e. methylenediphosphonic acid, with a biocompatible cation.

By the term "radioprotectant" is meant a compound which inhibits degradation reactions, such as redox processes, by trapping highly-reactive free radicals, such as oxygen-containing free radicals arising from the radiolysis of water. The radioprotectants of the present invention are suitably chosen from: ascorbic acid, *para*-aminobenzoic acid (i.e. 4-aminobenzoic acid), gentisic acid (i.e. 2,5-dihydroxybenzoic acid) and salts thereof with a biocompatible cation as described above.

By the term "antimicrobial preservative" is meant an agent which inhibits the growth of potentially harmful micro-organisms such as bacteria, yeasts or moulds. The antimicrobial preservative may also exhibit some bactericidal properties, depending on the dose. The main role of the antimicrobial preservative(s) of the present invention is to inhibit the growth of any such micro-organism in the pharmaceutical composition post-reconstitution, i.e. in the imaging agent itself. The antimicrobial preservative may, however, also optionally be used to inhibit the growth of potentially harmful micro-organisms in one or more components of the kit of the present invention prior to reconstitution. Suitable antimicrobial preservative(s) include: the parabens, i.e. methyl, ethyl, propyl or butyl paraben or mixtures thereof; benzyl alcohol; phenol; cresol; cetrimide and thiomersal. Preferred antimicrobial preservative(s) are the parabens.

The term "pH-adjusting agent" means a compound or mixture of compounds useful to ensure that the pH of the reconstituted kit is within acceptable limits (approximately pH 4.0 to 10.5) for human or mammalian administration. Suitable such pH-adjusting agents

include pharmaceutically acceptable buffers, such as tricine, phosphate or TRIS [i.e. *tris*(hydroxymethyl)aminomethane], and pharmaceutically acceptable bases such as sodium carbonate, sodium bicarbonate or mixtures thereof. When the MSRA antagonist-chelating agent conjugate is employed in acid salt form, the pH adjusting agent may optionally be provided in a separate vial or container, so that the user of the kit can adjust the pH as part of a multi-step procedure.

By the term "filler" is meant a pharmaceutically acceptable bulking agent which may facilitate material handling during production and lyophilisation. Suitable fillers include inorganic salts such as sodium chloride, and water soluble sugars or sugar alcohols such as sucrose, maltose, mannitol or trehalose.

A fifth aspect of the present invention is the use of the pharmaceutical composition of the invention for the diagnostic imaging of CVD. Preferably, the pharmaceutical composition of the invention may be used in the diagnostic imaging of atherosclerotic plaques, coronary artery disease, thrombosis, transient ischaemia or renal disease. Most preferably, the pharmaceutical composition of the invention may be used in the diagnostic imaging of atherosclerotic plaques. An especially preferred use of the pharmaceutical composition of the invention is for the diagnostic imaging of unstable atherosclerotic plaques.

A further use of the pharmaceutical composition of the invention is in the diagnostic imaging of neurological diseases in which microglial cells are involved, such as Alzheimer's disease, multiple sclerosis, Parkinson's disease and encephalitis.

Brief Description of the Figures

Figure 1 illustrates the general synthetic route used to obtain the sulphonamidobenzamide compounds of the invention.

Figure 2 illustrates the synthetic route used to obtain precursor 1 and the ^{99m}Tc-labelled imaging agent 1.

Figure 3 illustrates the chemical structures of precursor 2 and precursor 3, both of which are suitable for labelling with ^{99m}Tc.

Figure 4 illustrates the synthetic route used to obtain precursor 4 and the radioiodinated imaging agent 2.

Figure 5 illustrates the synthetic route used to obtain precursor 5 and the ^{18}F-labelled imaging agent 3.

Figure 6 shows competition of various agents for binding of ^{125}I-imaging agent 2 to THP-1 and CHO-SRA cells. The various agents are (A) non-radioactive imaging agent 2 (B) AcLDL and (C) OxLDL.

Figure 7 shows the binding of ^{125}I-imaging agent 2 to THP-1 Cells using a spiked assay format.

Figure 8 shows the biodistribution of ^{125}I-imaging agent 2 in rats over a period of 4 hours post injection (p.i.).

Brief Description of the Examples

Various embodiments of the invention are described in the following non-limiting examples.

Example 1 relates to the synthesis of chelating agent 1, which is then used in the preparation of precursor 1 in Example 2. Precursor 1 is a compound suitable for the attachment of a metal ion, preferably ^{99m}Tc, the attachment of which is described in Example 5. Examples 3 and 4 describe the synthesis of precursors 2 and 3, both suitable for labelling with ^{99m}Tc.

Example 6 describes the synthesis of precursor 4, a compound which is suitable for straightforward substitution with radiohalogen. The process of radioiodinating precursor 4 to form imaging agent 2 is described in Example 7.

Example 8 describes the synthesis of precursor 5, which is suitable for radiofluorination. Example 9 describes a method of preparing the ^{18}F compound from precursor 5.

Examples 10 and 11 describe how the non-radioactive reference versions of imaging agents 2 and 3 were prepared. These non-radioactive versions were used in the scavenger receptor binding assay because their binding characteristics would be identical to the radioiodinated imaging agents 2 and 3.

Example 12 outlines the method used to assess the binding characteristics of compounds of the invention. IC50 values of <40μM for were found in this binding assay for the non radioactive versions of imaging agents 2 and 3.

Example 13 and 14 describe the cell binding assay used to evaluate binding of ^{125}I imaging agent 2 to cells expressing MSRA. In example 13, ^{125}I imaging agent 2 was found to bind to MSRA present on THP-1 and CHO-SRA cells. Saturation binding of ^{125}I-imaging agent 2 to THP-1 cells fit to and was analysed by a one site binding curve, a K_d of 6.74μM was calculated. Therefore in contrast to the competition data, ^{125}I-imaging agent 2 appears to bind with μM affinity. In example 14, binding of ^{125}I imaging agent 2 was competed by cold ^{127}I imaging agent 2 and AcLDL demonstrating specific binding of the agent to MSRA.

Example 15 describes the *in vivo* characteristics of ^{125}I imaging agent 2 in a mouse tumour model. Results showed minimal de-iodination *in vivo*, approximately 20% up to 4 hours p.i. Blood retention was high throughout with a slow rate of excretion, with higher GI excretion (Figures 8A and 8B). Uptake of ^{125}I imaging agent 2 into tumour tissue was initially low but increased to peak at 60 minutes p.i. and remained constant thereon. Increased ^{125}I imaging agent 2 at tumour site was most likely specific as blood

retention decreased from 5-60 minutes p.i. Good tumour to muscle ratios were obtained, with optimal ratio seen at 60 minutes p.i. (Figure 8A).

Example 1: Synthesis of chelating agent 1

Step 1(a): 3(methoxycarbonylmethylene)glutaric acid dimethylester

Carbomethoxymethylenetriphenylphosphorane (167g, 0.5mol) in toluene (600ml) was treated with dimethyl 3-oxoglutarate (87g, 0.5mol) and the reaction heated to 100°C on an oil bath at 120°C under an atmosphere of nitrogen for 36h. The reaction was then concentrated *in vacuo* and the oily residue triturated with 40/60 petrol ether/diethylether 1:1, 600ml. Triphenylphosphine oxide precipitated out and the supernatant liquid was decanted/filtered off. The residue on evaporation *in vacuo* was Kugelrohr distilled under high vacuum Bpt (oven temperature 180-200°C at 0.2torr) to give 3(methoxycarbonylmethylene)glutaric acid dimethylester in 89.08g, 267mM, 53%.

NMR $^{1}H(CDCl_3)$: δ 3.31 (2H, s, CH_2), 3.7(9H, s, 3xOCH_3), 3.87 (2H, s, CH_2), 5.79 (1H, s, =CH,) ppm.

NMR $^{13}C(CDCl_3)$, δ 36.56,CH_3, 48.7, 2xCH_3, 52.09 and 52.5 (2xCH_2); 122.3 and 146.16 C=CH; 165.9, 170.0 and 170.5 3xCOO ppm.

Step 1(b): Hydrogenation of 3-(methoxycarbonylmethylene)glutaric acid dimethylester.

3(methoxycarbonylmethylene)glutaric acid dimethylester (89g, 267mmol) in methanol (200ml) was shaken with (10% palladium on charcoal: 50% water) (9 g) under an atmosphere of hydrogen gas (50 psi) for (30h). The solution was filtered through kieselguhr and concentrated in vacuo to give 3-(methoxycarbonylmethyl)glutaric acid dimethylester as an oil yield (84.9g, 94 %).

NMR $^{1}H(CDCl_3)$, δ (12H, m, 4xCH_3), (2H, m, 2xCH_2) (1H, hextet, CH) 3.7 (1H, doublet, CH), (8H, 2 quartets, 4xCH_2O).

NMR $^{13}C(CDCl_3)$, δ and $2xCH_3$, CH, 2xCH2, CH; and $2xCH_2$-O, 168.2 and 171.5 2xCOO.

Step 1(c): Reduction and esterification of trimethyl ester to the triacetate.

Under an atmosphere of nitrogen in a 3 necked 2L round bottomed flask lithium aluminium hydride (20g, 588mmol) in tetrahydrofuran (400ml) was treated cautiously with tri(methyloxycarbonylmethyl)methane (40g, 212mmol) in tetrahydrofuran (200ml) over 1h. A strongly exothermic reaction occurred, causing the solvent to reflux strongly. The reaction was heated on an oil bath at 90°C at reflux for 3 days. The reaction was quenched by the cautious dropwise addition of acetic acid (100ml) until the evolution of hydrogen ceased. The stirred reaction mixture was cautiously treated with acetic anhydride solution (500ml) at such a rate as to cause gentle reflux. The flask was equipped for distillation and stirred and then heating at 90°C (oil bath temperature) to distil out the tetrahydrofuran. A further portion of acetic anhydride (300ml) was added, the reaction returned to reflux configuration and stirred and heated in an oil bath at 140°C for 5h. The reaction was allowed to cool and filtered. The aluminium oxide precipitate was washed with ethyl acetate and the combined filtrates concentrated on a rotary evaporator at a water bath temperature of 50°C *in vacuo* (5 mmHg) to afford an oil. The oil was taken up in ethyl acetate (500ml) and washed with saturated aqueous potassium carbonate solution. The ethyl acetate solution was separated, dried over sodium sulphate, and concentrated *in vacuo* to afford an oil. The oil was Kugelrohr distilled in high vacuum to give *tris*(2-acetoxyethyl)methane (45.313g, 95.9% yield, 0.165 mol) as an oil. Bp. 220 at 0.1 mmHg.

NMR $^{1}H(CDCl_3)$, δ 1.66(7H, m, $3xCH_2$, CH), 2.08(1H, s, $3xCH_3$); 4.1(6H, t $3xCH_2O$).

NMR $^{13}C(CDCl_3)$, δ 20.9, CH_3; 29.34, CH; 32.17, CH_2; 62.15, CH_2O; 171, CO.

Step 1(d): Removal of Acetate groups from the triacetate.

Tris(2-acetoxyethyl)methane (45.3g, 165mM) in methanol (200ml) and 880 ammonia (100ml) was heated on an oil bath at 80°C for 2 days. The reaction was treated with a further portion of 880 ammonia (50ml) and heated at 80°C in an oil bath for 24h. A further portion of 880 ammonia (50ml) was added and the reaction heated at 80°C for 24h. The reaction was then concentrated *in vacuo* to remove all solvents to give an oil. This was taken up into 880 ammonia (150ml) and heated at 80°C for 24h. The reaction was then concentrated *in vacuo* to remove all solvents to give an oil. Kugelrohr distillation gave acetamide bp 170-180 0.2mm. The bulbs containing the acetamide were washed clean and the distillation continued. *Tris*(2-hydroxyethyl)methane (22:53g, 152mmol, 92.1%) distilled at bp 220 °C 0.2mm.

NMR $^{1}H(CDCl_3)$, δ 1.45(6H, q, $3xCH_2$), 2.2(1H, quintet, CH); 3.7(6H, t $3xCH_2OH$); 5.5(3H, brs, 3xOH).

NMR $^{13}C(CDCl_3)$, δ 22.13, CH; 33.95, $3xCH_2$; 57.8, $3xCH_2OH$.

Step 1(e): Conversion of the triol to the *tris*(methanesulphonate).

To an stirred ice-cooled solution of *tris*(2-hydroxyethyl)methane (10g, 0.0676mol) in dichloromethane (50ml) was slowly dripped a solution of methanesulphonyl chloride (40g, 0.349mol) in dichloromethane (50ml) under nitrogen at such a rate that the temperature did not rise above 15°C. Pyridine (21.4g, 0.27mol, 4eq) dissolved in dichloromethane (50ml) was then added drop-wise at such a rate that the temperature did not rise above 15°C, exothermic reaction. The reaction was left to stir at room temperature for 24h and then treated with 5N hydrochloric acid solution (80ml) and the layers separated. The aqueous layer was extracted with further dichloromethane (50ml) and the organic extracts combined, dried over sodium sulphate, filtered and concentrated *in vacuo* to give *tris*(2-(methylsulphonyloxy)ethyl)methane contaminated with excess methanesulphonyl chloride. Theoretical yield was 25.8g.

NMR $^{1}H(CDCl_3)$, δ 4.3 (6H, t, $2xCH_2$), 3.0 (9H, s, $3xCH_3$), 2 (1H, hextet, CH,), 1.85 (6H, q, $3xCH_2$).

Step 1(f): Preparation of 1,1,1-*tris*(2- azidoethyl)methane.

A stirred solution of *tris*(2-(methylsulphonyloxy)-ethyl)methane [from step 1(e), contaminated with excess methylsulphonyl chloride] (25.8g, 67mmol, theoretical) in dry DMF (250ml) under nitrogen was treated with sodium azide (30.7g, 0.47mol) portion-wise over 15 minutes. An exotherm was observed and the reaction was cooled on an ice bath. After 30 minutes, the reaction mixture was heated on an oil bath at 50°C for 24h. The reaction became brown in colour. The reaction was allowed to cool, treated with dilute potassium carbonate solution (200ml) and extracted three times with 40/60 petrol ether/diethylether 10:1 (3x150ml). The organic extracts were washed with water (2x150ml), dried over sodium sulphate and filtered. Ethanol (200ml) was added to the petrol/ether solution to keep the triazide in solution and the volume reduced *in vacuo* to **no less than** 200ml. Ethanol (200ml) was added and reconcentrated *in vacuo* to remove the last traces of petrol leaving **no less than** 200ml of ethanolic solution.

CARE: DO NOT REMOVE ALL THE SOLVENT AS THE AZIDE IS POTENTIALLY EXPLOSIVE AND SHOULD BE KEPT IN DILUTE SOLUTION AT ALL TIMES.

NMR $^1H(CDCl_3)$, δ 3.35 (6H, t, $3xCH_2$), 1.8 (1H, hextet, CH,), 1.6 (6H, q, $3xCH_2$).

Step 1(g): Preparation of 1,1,1-*tris*(2-aminoethyl)methane.

Tris(2-azidoethyl)methane (15.06g, 0.0676 mol), (assuming 100% yield from previous reaction) in ethanol (200ml) was treated with 10% palladium on charcoal (2g, 50% water) and hydrogenated for 12h. The reaction vessel was evacuated every 2 hours to remove nitrogen evolved from the reaction and refilled with hydrogen. A sample was taken for NMR analysis to confirm complete conversion of the triazide to the triamine. Caution: unreduced azide could explode on distillation. The reaction was filtered through a celite pad to remove the catalyst and concentrated in vacuo to give *tris*(2-aminoethyl)methane as an oil. This was further purified by Kugelrohr distillation bp.180–200°C at 0.4mm/Hg to give a colourless oil (8.1g, 55.9 mmol, 82.7% overall yield from the triol).

NMR $^{1}H(CDCl_3)$, 2.72 (6H, t, $3xCH_2N$), 1.41 (H, septet, CH), 1.39 (6H, q, $3xCH_2$).

NMR $^{13}C(CDCl_3)$, δ 39.8 (CH_2NH_2), 38.2 (CH_2.), 31.0 (CH).

Step 1(h): Synthesis of bis[N-(1,1-dimethyl-2-N-hydroxyimine propyl)2-aminoethyl]-(2-aminoethyl) methane (chelating agent 1).

To a solution of *tris*(2-aminoethyl)methane (4.047g, 27.9mmol) in dry ethanol (30ml) was added potassium carbonate anhydrous (7.7g, 55.8mmol, 2eq) at room temperature with vigorous stirring under a nitrogen atmosphere. A solution of 3-chloro-3-methyl-2-nitrosobutane (7.56g, 55.8mol, 2eq) was dissolved in dry ethanol (100ml) and 75ml of this solution was dripped slowly into the reaction mixture. The reaction was followed by TLC on silica run in dichloromethane, methanol, concentrated (0.88sg) ammonia; 100/30/5 and the TLC plate developed by spraying with ninhydrin and heating. The mono, di and tri alkylated products were seen with RF's increasing in that order. Analytical HPLC was run using RPR reverse phase column in a gradient of 7.5-75% acetonitrile in 3% aqueous ammonia. The reaction was concentrated *in vacuo* to remove the ethanol and resuspended in water (110ml). The aqueous slurry was extracted with ether (100ml) to remove some of the trialkylated compound and lipophilic impurities leaving the mono and desired dialkylated product in the water layer. The aqueous solution was buffered with ammonium acetate (2eq, 4.3g, 55.8mmol) to ensure good chromatography. The aqueous solution was stored at 4°C overnight before purifying by automated preparative HPLC.

Yield (2.2g, 6.4mM, 23%).

Mass spec; Positive ion 10 V cone voltage. Found: 344; calculated M+H= 344.

NMR $^{1}H(CDCl_3)$, δ 1.24(6H, s, $2xCH_3$), 1.3(6H, s, $2xCH_3$), 1.25-1.75(7H, m, $3xCH_2$,CH), (3H, s, $2xCH_2$), 2.58 (4H, m, CH_2N), 2.88(2H, t CH_2N_2), 5.0 (6H, s, NH_2 , 2xNH, 2xOH).

NMR ^{1}H $((CD_3)_2SO)$ δ1.1 4xCH; 1.29, $3xCH_2$; 2.1 (4H, t, $2xCH_2$);

NMR $^{13}C((CD_3)_2SO)$, δ 9.0 ($4xCH_3$), 25.8 ($2xCH_3$), 31.0 $2xCH_2$, 34.6 CH_2, 56.8 $2xCH_2N$; 160.3, C=N.

HPLC conditions: flow rate 8ml/min using a 25mm PRP column
A=3% ammonia solution (sp.gr = 0.88) /water.
B=Acetonitrile

Time	%B
0	7.5
15	75.0
20	75.0
22	7.5
30	7.5

Load 3ml of aqueous solution per run, and collect in a time window of 12.5-13.5 min.

Example 2: Attachment of chelating agent 1 to 4-carboxy-N-(4-bromophenyl)-2-(4-chlorophenylsulfonylamido) benzamide to form precursor 1

Chelating agent 1 was attached to 4-carboxy-N-(4-bromophenyl)-2-(4-chlorophenylsulfonylamido) benzamide by means of Step 1 of the reaction scheme depicted in Figure 2.

To a solution of 4-carboxy-N-(4-bromophenyl)-2-(4-chlorophenylsulfonylamido) benzamide (1mg) in dichloromethane (2ml) at room temperature was added 4 equivalents of TBTU and 1.1 equivalent of chelating agent 1 of Formula (V), and 3 equivalents of N,N-diisopropylethylamine (DIEA) under a nitrogen atmosphere for 24 hours. The crude mixture was purified by HPLC.

Mass Spectrometry analysis: ES [M+H] *m/z* 836.

Example 3: Attachment of chelating agent 1 to 4-bromo-N-(4-bromophenyl)-2-(4-carboxyphenylsulfonylamido) benzamide to form precursor 2

The method described in Example 2 was used to attach chelating agent 1 to 4-bromo-N-(4-bromophenyl)-2-(4-carboxyphenylsulfonylamido) benzamide to form precursor 2, which is illustrated in Figure 3.

Example 4: Attachement of chelating agent 1 to 4-bromo-N-(4-carboxyphenyl)-2-(4-chlorophenylsulfonylamido) benzamide to form precursor 3

The method described in Example 2 was used to attach chelating agent 1 to 4-bromo-N-(4-carboxyphenyl)-2-(4-chlorophenylsulfonylamido) benzamide to form precursor 3, which is illustrated in Figure 3.

Example 5: ^{99m}Tc labelling of precursor 1 to form imaging agent 1

Imaging agent 1 was prepared by labelling precursor 1 with ^{99m}Tc according to Step 2 of the reaction scheme depicted in Figure 2.

A $SnCl_2$/MDP solution was prepared by dissolving 10mg $SnCl_2$ and 90mg MDP in 100ml of nitrogen-purged saline. To 50µl 1mg/ml in methanol of precursor 1, was added; (1) 0.7ml methanol, (2) 0.5ml 0.1M sodium carbonate buffer, (3) 0.5ml 500MBq/ml TcO_4, and (4) 100µl of the $SnCl_2$/MDP solution. This reaction mixture was heated at 37°C for 30min to form imaging agent 1. The activity of solution was 185 MBq.

An ITLC (instant thin layer chromatography) using silica gel plates and a mobile phase of MeOH/(NH_4OAc 0.1M) 1:1 showed 3 % RHT (reduced hydrolysed technetium) at the origin. HPLC analysis showed 88% of imaging agent 1 to give an RCP of 85%. The retention time of imaging agent 1 was 16.6 min.

HPLC analyses was carried out using an Xterra RP18, 3.5μm, 4.6 x 150 mm column using an aqueous mobile phase (solvent A) of 0.06% NH_4OH and organic mobile phase (solvent B) of acetonitrile and a flow rate of 1ml/min. Typical gradients used were as follows: 0-5 min (10-30% B), 5-20 min (30% B), 20-21 min (30-100% B), 21-25 min (100% B) and 25-27 min (100-10% B).

Example 6: Synthesis of precursor 4

Step 1 of the reaction scheme depicted in Figure 4 is used to prepare precursor 4. 4-*n*-tributyltin aniline is coupled to 5-bromo-2-(4-chlorophenylsulfonamido) benzoic acid in DCM in the presence of 1.5 equivalents of triethylamine to give precursor 4.

Example 7: Radioiodination of precursor 4 to form imaging agent 2

Radioiodination of precursor 4 to form imaging agent 2 is carried out according to Step 2 of the scheme depicted in Figure 4.

10 μM of precursor 4 is reacted with 0.05 mL NaI solution [approx. 0.167 μM total radioiodine (I*)] in the presence of 0.4 mL DMF, 0.1 mL ammonium acetate buffer (pH 4, 0.2 M) and 0.05 mL Chloramine-T solution (0.22 μM). 0.5 mL H_2O is added after 5 minutes. The crude mixture is subsequently separated by HPLC to yield pure imaging agent 2.

Example 8: Synthesis of precursor 5

In Step 1 of the reaction scheme illustrated in Figure 5, 3-chloro-4-nitrobenzenesulfonic acid is reacted with $POCl_3$ to form 3-chloro-4-nitrobenzenesulfonyl chloride, which is reacted with N-(4-bromophenyl)-2-amino-5-bromobenzamide to form 4-bromo-N-(4-bromophenyl)-2-(3-chloro- 4-nitro- phenyl sulphonamido) benzamide (Step 2). The nitro group is then reduced with $SnCl_2.2H_2O$ to yield the amine (Step 3). The amine is then converted into the diazonium compound by treatment with nitrous acid (HONO) in Step 4 to form precursor 5.

Example 9: Synthesis of imaging agent 3

In Step 5 of the scheme illustrated in Figure 4 $^{18}F^-$ is reacted with the diazonium compound to give imaging agent 3.

Example 10: Preparation of non-radioactive imaging agent 2

A solution of *N*-(4-iodophenyl)-2-amino-5-bromobenzamide (3 mmol), 4-chlorobenzenesulfonyl chloride (3 mmol) and pyridine (12 mmol) in dichloromethane (20 ml) was stirred for 18 hours under a nitrogen atmosphere. The solvent was removed under vacuum and the residue dissolved in methanol. Purification was done by HPLC (C 18, 20-95% acetonitrile - 0.1% aqueous trifluoroacetic acid).

After HPLC purification the product gave the required ^{1}H NMR and MS analysis.

^{1}H NMR (CD_3OD) δ 7.32 (2H, d, J = 4.5 Hz), 7.38 (2H, d, J = 4.5 Hz), 7.52 (1H, d, J = 4.5 Hz), 7.59 (2H, d, J = 4.5 Hz), 7.65-7.70 (3H, m), 7.82 (1H, d, J = 1 Hz)

MS (ES-ve) m/z = 591.

Example 11: Preparation of non-radioactive imaging agent 3

A solution of *N*-(4-bromophenyl)-2-amino-5-bromobenzamide (3 mmol), 3-chloro-4-fluorobenzenesulfonyl chloride (3 mmol) and pyridine (12 mmol) in dichloromethane (20 ml) was stirred for 18 hours under a nitrogen atmosphere. The solvent was removed under vacuum and the residue dissolved in methanol. Purification was done by HPLC (C 18, 20-95% acetonitrile - 0.1% aqueous trifluoroacetic acid).

After HPLC purification the product gave the required ^{1}H NMR analysis.

^{1}H NMR (CD_3OD) δ7.20 (1H, t, *J* = 9 Hz), 7.46-7.59 (6H, m), 7.68 (1H, dd, *J* = 3 and 6 Hz), 7.80 (1H, dd, *J* = 2.4 and 4.8 Hz), 7.84 (1H, d, *J* = 1.1 Hz)

Example 12: Scavenger receptor binding assay

An assay was developed based on that described in Lysko *et al.* [1999 J. Pharmacol Exp Ther 289 (3); 1277-1285]. The cells used in the assay were either mouse J774.1 or human THP-1. J774.1 cells were seeded at $1x10^5$ cells/well/ml 24 hours prior to assay in Dulbecco's minimum essential medium containing penicillin/streptomycin, 2mM glutamine and 10% foetal bovine serum. THP-1 cells were seeded at $1x10^5$ cells/well/ml in RPMI-1640 medium containing penicillin/streptomycin, 2mM glutamine and 10% foetal bovine serum with 400ng/ml phorbol myristate acetate 4-6 days prior to assay. For the assay, the medium was decanted from the plates and they were washed with 1ml/well ice cold phosphate-buffered saline containing 2mg/ml BSA. Into the wells, add the following reagents (all in μl):

	NSB well	**Bo well**	**Assay well**
Assay buffer	100	150	100
Competing compound	-	-	50
acLDL (for NSB*)	50	-	-
[^{125}I]acLDL	50	50	50

*NSB = non-specific binding

The assay buffer was Dulbecco's minimal essential medium containing penicillin/streptomycin, 2mM glutamine and 2mg/ml bovine serum albumin. AcLDL was obtained from Biogenesis (Cat no. 5685-3404). [^{125}I]acLDL (Biogenesis, Cat. no. 5685-3502) used at 150,000cpm per well in assay (approx. 1.5μg/ml).

The plates were incubated for 3 hours at 37°C after which time the reagents were removed and the plates were washed with pre-chilled wash buffer (2: 0.15M NaCl, 50mM Tris-HCl, pH7.4). The plates were then incubated with pre-chilled wash buffer for 10 minutes on ice, and this step was then repeated. A further rapid wash was carried

out with a different wash buffer (0.15M NaCl, 50mM Tris-HCl, pH7.4) before adding 500µl NaOH for 30 minutes at room temperature. The well contents were transferred to Sarstedt tubes for radioactivity counting on a Wallac 1480 Wizard automatic gamma counter.

In order to assess cell coverage in the wells and show that cells were not lost during assay, 300µl of a 2% crystal violet stain in 95% methanol was added to the wells and incubated for 30 minutes at room temperature.

The IC50 for non-radioactive imaging agent 2 was 25.2µM, and chemically identical radioiodinated versions of the compound should produce similar values. The IC50 for non-radioactive imaging agent 3 was found to be 25.9µM, and the chemically identical ^{18}F labelled version of the compound should produce a similar value.

Example 13: Spiked saturation binding assay

Experiments were carried out on THP-1 cells. THP-1 cells (human monocyte ECACC) are suspension cells and were cultured in RPMI-1640 medium (Sigma, Cat no. R0883) containing penicillin/streptomycin (Sigma, Cat no. P4458), 2mM glutamine (Sigma, Cat no. G7513) and 10% foetal bovine serum (FBS; Sigma, Cat no. F-7524). Cells were routinely passaged at $2x10^5$ cells/ml in $162cm^2$ flasks. For assays with ^{125}I-acLDL, cells were seeded at $1x10^5$ cells/well/ml in 24 well plates 4-6 days prior to assay and 400ng/ml phorbol myristate acetate (PMA) added to differentiate THP-1 cells. Cells became adherent and expressed MSRA. For assays with ^{125}I imaging agent 2, THP-1 cells were seeded at 1.5×10^5 cells/well/ml in 24 well plates 4-6 days prior to assay with 400ng/ml PMA.

^{125}I imaging agent 2 was diluted to a concentration of 400,000 cpm/50µl in assay buffer. Non-radioactive imaging agent 2 was prepared at 1mg/ml stock in 12.5% DMSO in assay buffer according to the method described in Example 10. For non-specific binding (NSB) measurement, a 1:2 dilution of this stock was prepared.

For spiked samples, two series of non- radioactive imaging agent 2 dilutions were also set up. For series A, a starting concentration of 0.125mg/ml (or 0.031mg/ml per well) was prepared, and 15 further doubling dilutions were made. For series B, a starting concentration of 0.188mg/ml (or 0.047mg/ml per well) was prepared, and 15 further doubling dilutions were made.

Media was decanted from plates and wells washed in ice cold PBS at 1ml/well. PBS was removed by decanting and any residual liquid removed with a pipette tip.

Assay reagents were then added as below:

- 100µl assay buffer (except in wells without non-radioactive imaging agent 2 where 150µl assay buffer was added)
- 50µl non-radioactive imaging agent 2 (appropriate dilution)
- 50µl ^{125}I imaging agent 2

Plates were incubated for 3hr, 37°C. Assay reagents were removed with a pipette tip and wells washed twice with 1ml pre-chilled wash buffer 1. Plates were then incubated with 1ml/well pre-chilled wash buffer 1 for 10min on ice. Wash buffer was decanted and a further 10min incubation on ice with wash buffer 1 carried out. A rapid wash with 1ml/well pre-chilled wash buffer 2 on ice was then carried out. Trypan blue (200µl of a 1:5 dilution in PBS) was then added to each well to check for toxicity. 500µl 0.1M NaOH was then added and plates left for 30min, R.T. Well contents were then transferred to Sarstedt tubes and counted on a Wallac Wizard.

Example 14: Cell competition assay with ^{125}I-imaging agent 2

Experiments were carried out in THP-1 and CHO-SRA cells to assess competition for binding of ^{125}I imaging agent 2 by acLDL, oxLDL and non-radioactive imaging agent 2.

THP-1 cells were cultured as described in Example 13 above.

CHO-SRA cells are adherent hamster ovary cells expressing human SR-A and were cultured in Hams F-12 medium containing penicillin/streptomycin, 2mM glutamine, HEPES buffer (7ml per 500ml media), 3% filter-sterilised lipoprotein-deficient serum (LPDS; Sigma cat. no. S5394), 40μM compactin/mevastatin (Sigma cat. no. M2537), 250μM mevalonic acid lactone (Sigma cat. no. M4667), and 3μg/ml acetylated LDL (AcLDL; Biogenesis, Cat no. 5685-3404). For assays with ^{125}I-acLDL, cells were seeded at 1x10^5 cells/well/ml in 24 well plates 24 hours prior to assay. For assays with ^{125}I imaging agent 2, CHO-SRA cells were seeded at 1.5 x 10^5 cells/well/ml in 24 well plates 24 hours prior to assay.

In THP-1 cells, non-radioactive imaging agent 2 competed for binding of ^{125}I imaging agent 2 with two site kinetics (Figure 6A). Mean IC_{50} values of 79 ± 20nM (n=3) and 14.5 ± 5.8μM (n=3) were obtained. However in CHO-SRA cells one site competition was observed with a mean IC_{50} value of 30 ± 12μM (n=3).

AcLDL (Figure 6B) and oxLDL (Figure 6C) both displayed one site competition for binding of ^{125}I imaging agent 2 in THP-1 and CHO-SRA cells. Mean IC_{50} values for acLDL were ~281μg/ml (n=3) in THP-1 cells and 192μg/ml (n=2) in CHO-SRA cells. Mean IC_{50} values for oxLDL were ~369μg/ml (n=2) in THP-1 cells and 112μg/ml (n=1) in CHO-SRA cells.

Example 15: In vivo assessment of imaging agent 2

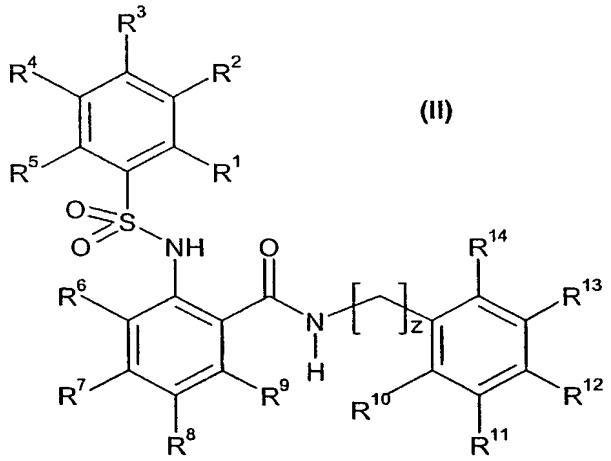
J774 cells are mouse macrophage cells that express MSRA and MSRB (macrophage scavenger receptor B). A J774 tumour model was utilised for screening of MRSA targeted vectors. These cells have been utilised to generate *in vivo* tumours in BALB/C mice (Ralph *et al*, 1975 J Immunol. 114(2) pp898-905). J774 tumours were inoculated subcutaneously in BALB/C male mice with biodistribution studies performed 24-28 days post inoculation.

0.1ml of ^{125}I imaging agent 2 was injected intravenously as bolus *via* the tail vein of tumoured mice, with animals euthanised at 5, 30, 60, 120 and 240 minutes p.i.

Claims

1) An imaging agent which comprises a synthetic MSRA antagonist labelled with an imaging moiety, wherein the synthetic MSRA antagonist is a sulphonamidobenzamide compound, and wherein the imaging moiety can be detected externally in a non-invasive manner following administration of said labelled synthetic MSRA antagonist to the mammalian body *in vivo*.

2) The imaging agent of claim 1 wherein the sulphonamidobenzamide compound is of Formula (II):



(II)

wherein;

z is 0, 1 or 2;

R^1-R^{14} are independently R groups, where R is;

hydrogen, hydroxy, carboxy, C_{1-6} alkyl, nitro, cyano, amino, halogen, C_{6-14} aryl, C_{2-7} alkenyl, C_{2-7} alkynyl, C_{1-6} acyl, C_{7-15} aroyl, C_{2-7} carboalkoxy, C_{2-15} carbamoyl, C_{2-15} carbamyl, C_{1-6} alkysulphinyl, C_{6-14} arylsulphinyl, C_{6-12} arylalkylsulphinyl, C_{1-6} alkylsulphonyl, C_{6-14} arylsulphonyl, C_{6-12} arylalkylsulphonyl, sulphamyl, C_{6-14} arylsulphonamido or C_{1-6} alkylsulphonamido.

3) The imaging agent of claim 2 wherein each R^1 to R^{14} is chosen from:

an imaging moiety, hydrogen, C_{1-6} alkyl, hydroxy, carboxy, amino or halogen.

4) The imaging agent of claims 2 and 3 wherein one of R^2, R^3, R^7, R^8 and R^{12} in Formula (II) is an imaging moiety, and the remaining R^2, R^3, R^7, R^8 and R^{12} groups are independently selected from hydrogen, C_{1-6} alkyl, carboxy, or a halogen selected from chlorine, bromine, fluorine or iodine.

5) The imaging agent of claims 2-4 wherein R^3, R^8 and R^{12} are each independently a halogen selected from chlorine, bromine, fluorine or iodine.

6) The imaging agent of claims 1-5 wherein said imaging moiety is selected from:
   (i) a radioactive metal ion;
   (ii) a paramagnetic metal ion;
   (iii) a gamma-emitting radioactive halogen;
   (iv) a positron-emitting radioactive non-metal;
   (v) a hyperpolarised NMR-active nucleus;
   (vi) a reporter suitable for *in vivo* optical imaging;
   (vii) a β-emitter suitable for intravascular detection.

7) The imaging agent of claim 6, wherein the radioactive metal ion is a gamma emitter or a positron emitter.

8) The imaging agent of claim 7, wherein the radioactive metal ion is selected from ^{99m}Tc, ^{94m}Tc, ^{111}In, ^{113m}In, ^{64}Cu, ^{67}Cu, ^{67}Ga, ^{68}Ga, ^{48}V, ^{52}Fe and ^{55}Co.

9) The imaging agent of claim 6, wherein the paramagnetic metal ion is selected from paramagnetic ions of Gd, Mn and Fe.

10) The imaging agent of claim 7, wherein the paramagnetic metal ion is Gd(III).

11)The imaging agent of claim 6, wherein the gamma-emitting radioactive halogen is a radioactive isotope of iodine.

12)The imaging agent of claim 11, wherein the radioactive isotope of iodine is chosen from ^{123}I or ^{131}I.

13)The imaging agent of claim 6, wherein the positron-emitting radioactive non-metal is selected from ^{11}C, ^{13}N, ^{15}O, ^{17}F, ^{18}F, ^{124}I, ^{75}Br and ^{76}Br.

14)The imaging agent of claim 13, wherein the positron-emitting radioactive non-metal is ^{18}F.

15)The imaging agent of claim 6 wherein the hyperpolarised NMR-active nucleus is selected from ^{13}C, ^{15}N, ^{19}F, ^{29}Si and ^{31}P.

16)The imaging agent of claim 15 wherein the hyperpolarized NMR-active nucleus is ^{13}C.

17)The imaging agent of claims 6-10, wherein the imaging moiety is a radioactive or a paramagnetic metal ion and the metal ion is attached to the MSRA antagonist as part of a metal complex to form a conjugate of Formula (III):

[{MSRA antagonist}-$(L)_x$]$_y$-[metal complex] (III)

wherein:

-$(L)_x$- is a linker group wherein each L is independently -CZ_2-, -CZ=CZ-, -C≡C-, -CZ_2CO_2-, -CO_2CZ_2-, -NZCO-, -CONZ-, -NZ(C=O)NZ-, -NZ(C=S)NZ-, -SO_2NZ-, -$NZSO_2$-, -CZ_2OCZ_2-, -CZ_2SCZ_2-, -CZ_2NZCZ_2-, a C_{4-8} cycloheteroalkylene group, a C_{4-8} cycloalkylene group, a C_{5-12} arylene group, a C_{3-12} heteroarylene group, an amino acid or a monodisperse polyethyleneglycol (PEG) building block;

Z is independently chosen from H, C_{1-4} alkyl, C_{2-4} alkenyl, C_{2-4} alkynyl, C_{1-4} alkoxyalkyl or C_{1-4} hydroxyalkyl;

x is an integer of value 0 to 10; and

y is 1, 2 or 3.

18)The imaging agent of claim 17 wherein the metal complex is a coordination complex of the radioactive metal ion or the paramagnetic metal ion with one or more ligands.

19)The imaging agent of claim 18 wherein said one or more ligands are chelating agents selected from diaminedioximes, N_3S ligands, N_2S_2 ligands, N_4 ligands and N_2O_2 ligands.

20)An imaging agent precursor of Formula (IIIa):

[{MSRA antagonist}-$(L)_x$]$_y$-[ligand] **(IIIa)**

wherein:
$(L)_x$ is a linker group wherein L is as defined in claim 17;
x is an integer of value 0 to 10; and
y is 1, 2 or 3.

21)A pharmaceutical composition comprising the imaging agent of claims 1-19 together with a biocompatible carrier, in a form suitable for mammalian administration.

22)The pharmaceutical composition of claim 21 for use in the diagnostic imaging of cardiovascular disease.

23)The pharmaceutical composition of claims 21 and 22 for use in the diagnostic imaging of atherosclerotic plaques, coronary artery disease, thrombosis, transient ischaemia or renal disease.

24)The pharmaceutical composition of claim 23 for use in the diagnostic imaging of atherosclerotic plaques.

25)The pharmaceutical composition of claim 24 for use in the diagnostic imaging of unstable atherosclerotic plaques.

26)A kit for the preparation of the pharmaceutical composition of any of claims 21-27 comprising a precursor of the imaging agent of any of claims 1-19.

27)The kit of claim 26 wherein said precursor is of Formula (IIIa) of claim 20.

28)The kit of claim 27 wherein the preparation of said pharmaceutical composition comprises reaction of a radioactive metal ion or a paramagnetic metal ion with the precursor of Formula (IIIa).

29)The kit of claim 28 wherein the radioactive metal ion is selected from ^{99m}Tc, ^{111}In, ^{64}Cu, ^{67}Cu, ^{67}Ga and ^{68}Ga.

30)The kit of claims 28 and 29 wherein the radioactive metal ion is ^{99m}Tc.

31)The kit of claim 28 wherein the paramagnetic metal ion is selected from Gd, Mn and Fe.

32)The kit of claim 31 wherein the paramagnetic metal ion is Gd(III).

33)Use of the imaging agent of claims 1-20 for the diagnostic imaging of cardiovascular disease.

34)The use of claim 33 wherein the cardiovascular disease is atherosclerosis.

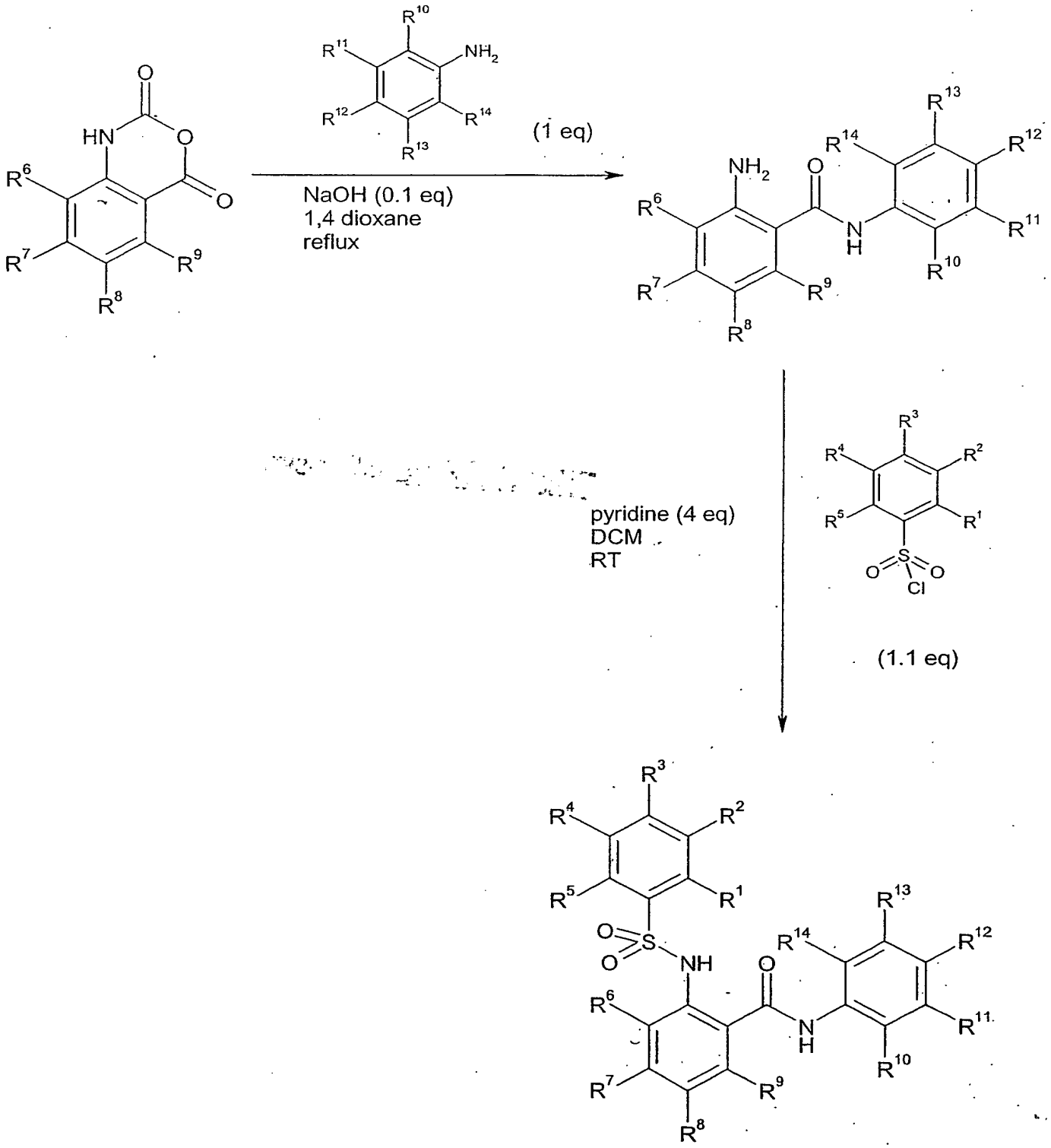
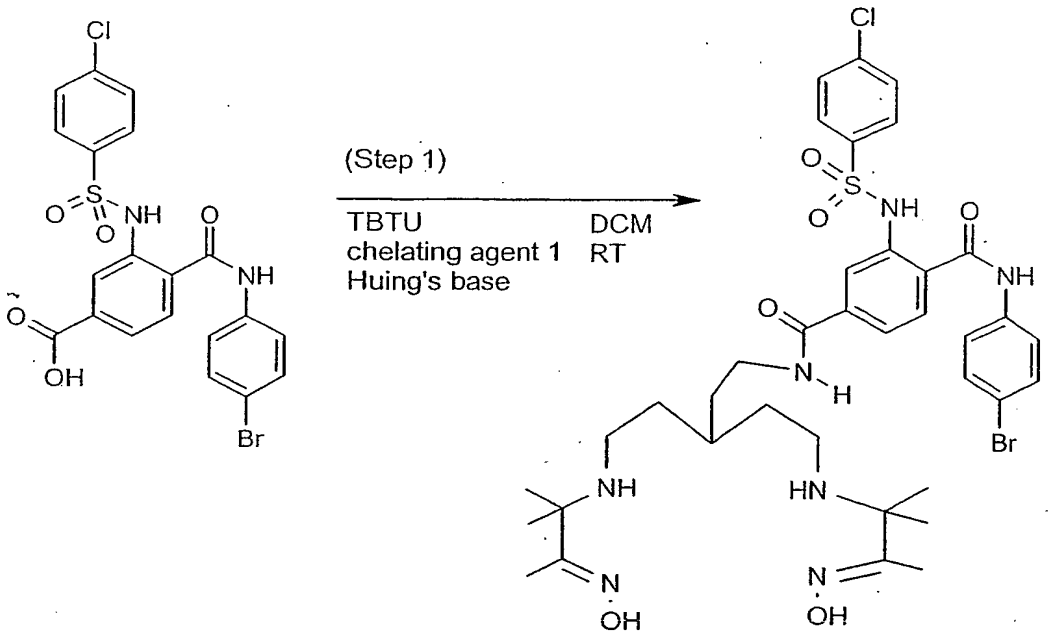


Figure 1


(Step 1)
TBTU
chelating agent 1
Huing's base
DCM
RT
precursor 1

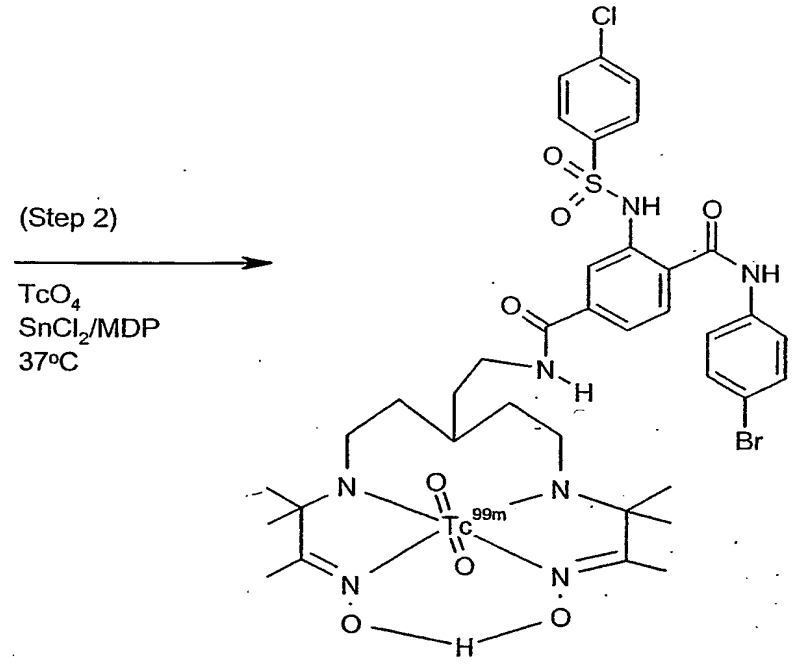
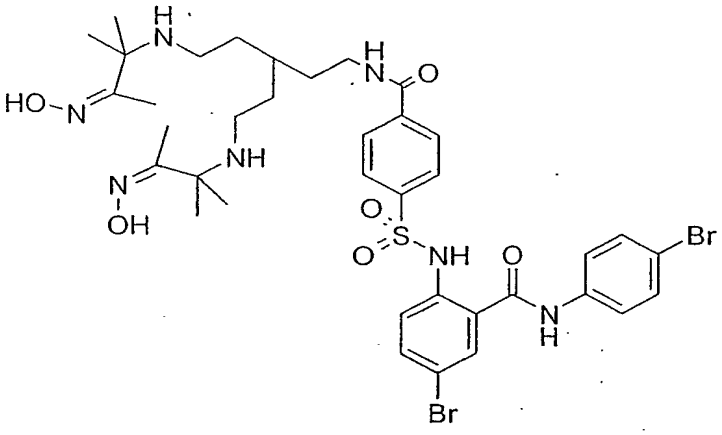
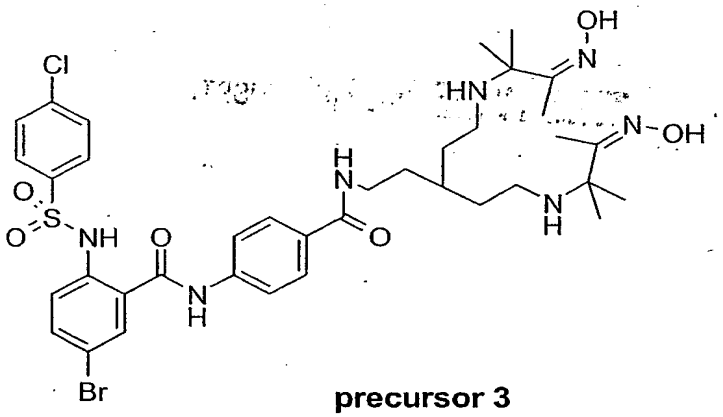

(Step 2)
TcO_4
$SnCl_2$/MDP
37°C
imaging agent 1

Figure 2



precursor 2



precursor 3

Figure 3

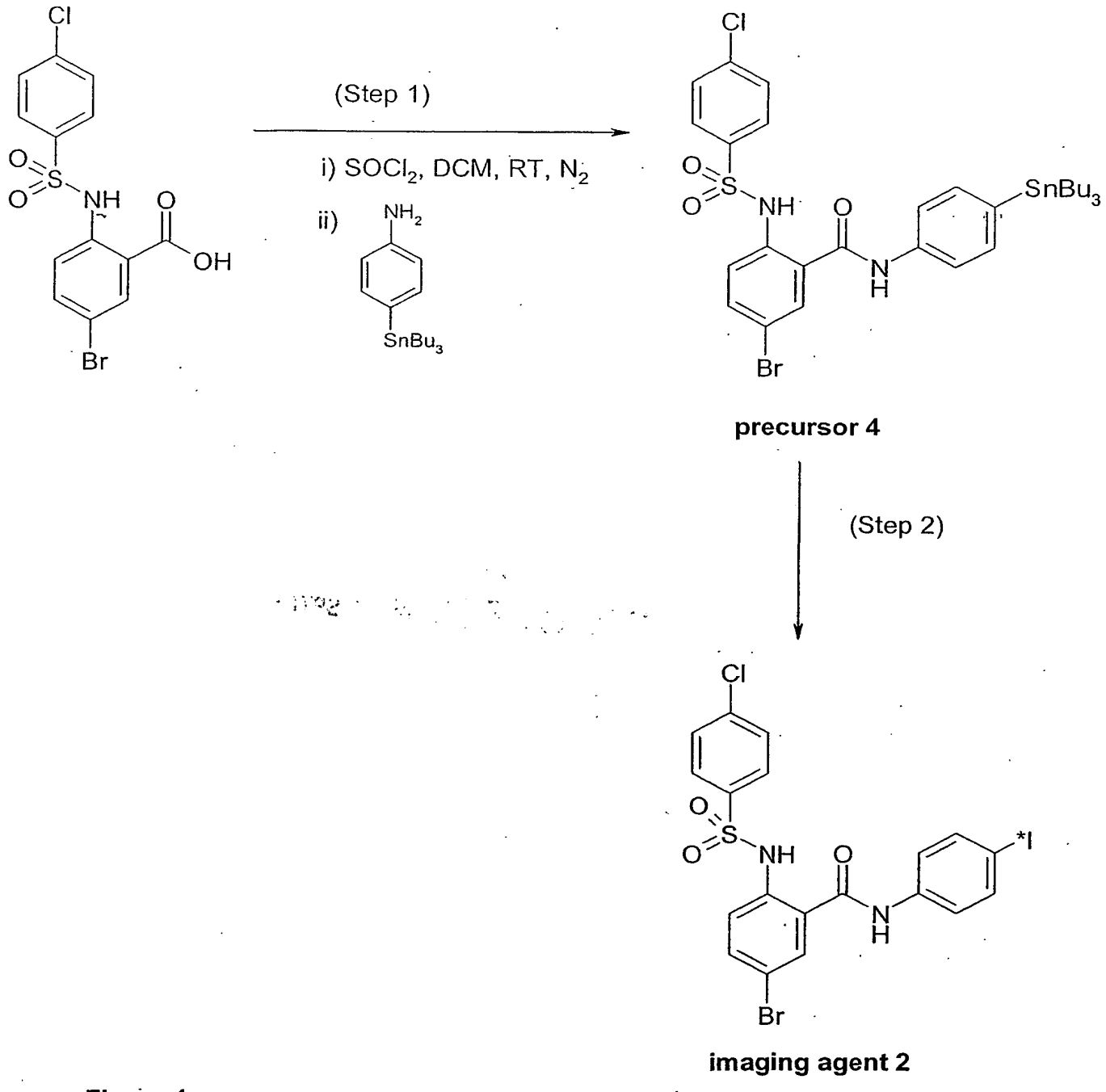

Cl
O
S
O
NH
O
OH
Br
(Step 1)
i) SOCl2, DCM, RT, N2
ii)
NH2
SnBu3
Cl
O
S
O
NH
O
N
H
SnBu3
Br
precursor 4
(Step 2)
Cl
O
S
O
NH
O
N
H
*I
Br
imaging agent 2

Figure 4

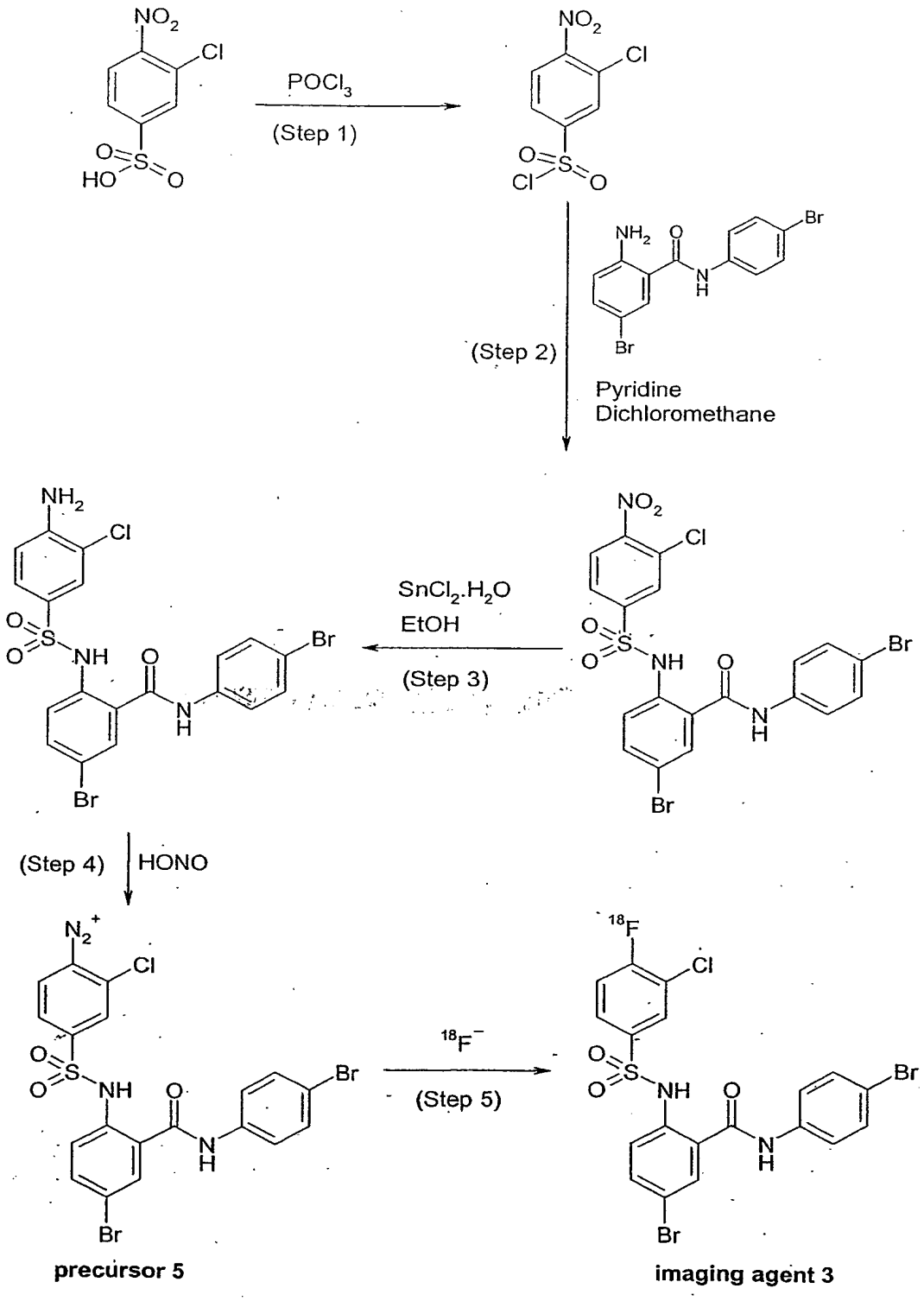

NO2
Cl
POCl3
(Step 1)
HO
O
S
O
Cl
NH2
Br
(Step 2)
Pyridine
Dichloromethane
SnCl2.H2O
EtOH
(Step 3)
(Step 4)
HONO
N2+
18F
18F−
(Step 5)
precursor 5
imaging agent 3

Figure 5

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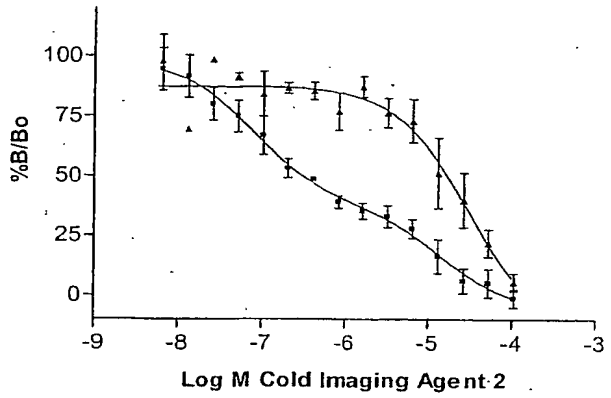
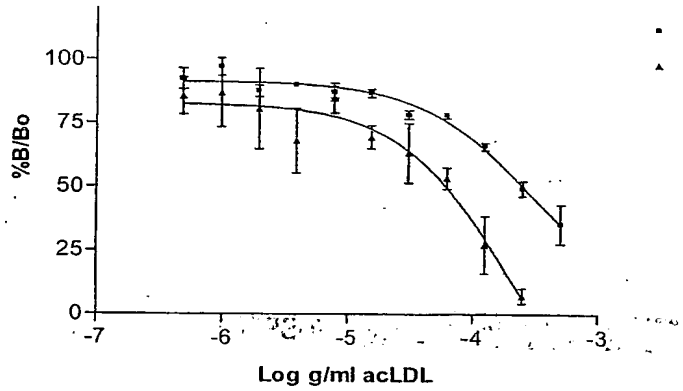

100
75
50
25
0
%B/Bo
-9
-8
-7
-6
-5
-4
-3
Log M Cold Imaging Agent 2
THP-1
CHO-SRA

A


100
75
50
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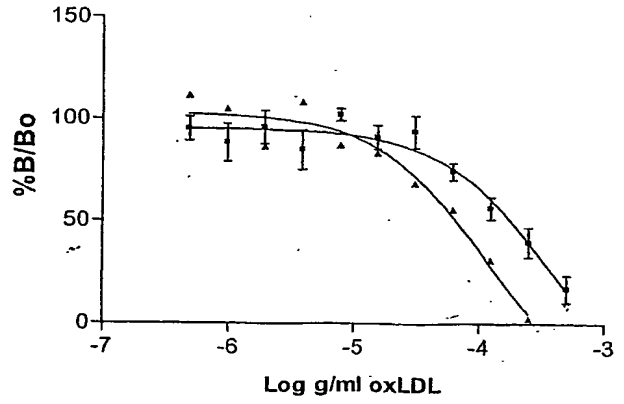
0
%B/Bo
-7
-6
-5
-4
-3
Log g/ml acLDL
THP acLDL
CHO acLDL

B


150
100
50
0
%B/Bo
-7
-6
-5
-4
-3
Log g/ml oxLDL
THP oxLDL
CHO oxLDL

C

Figure 6

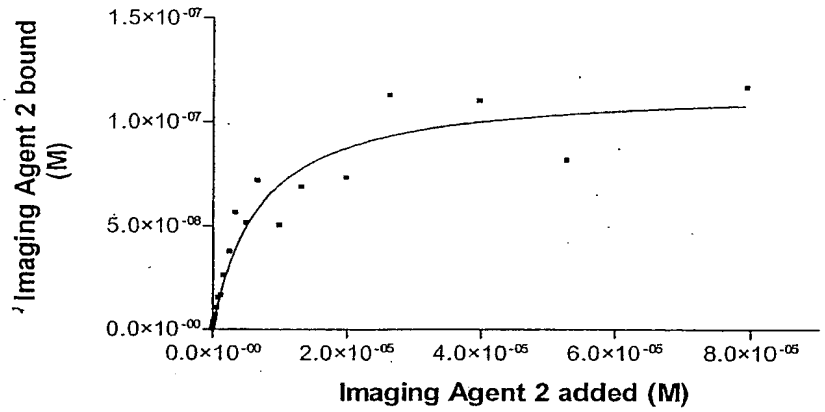
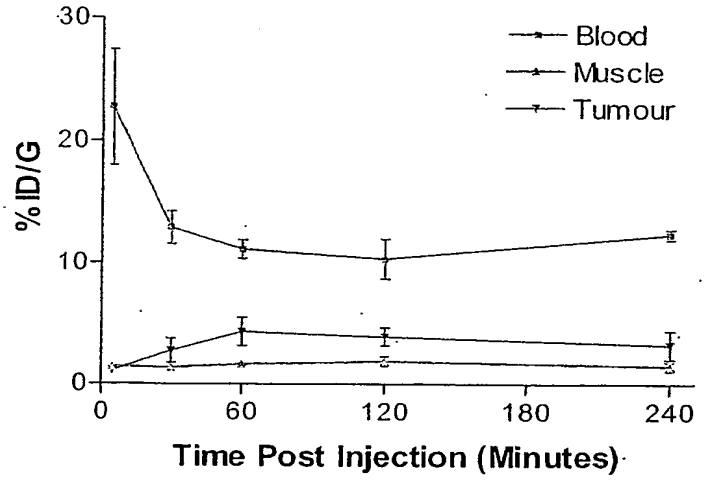
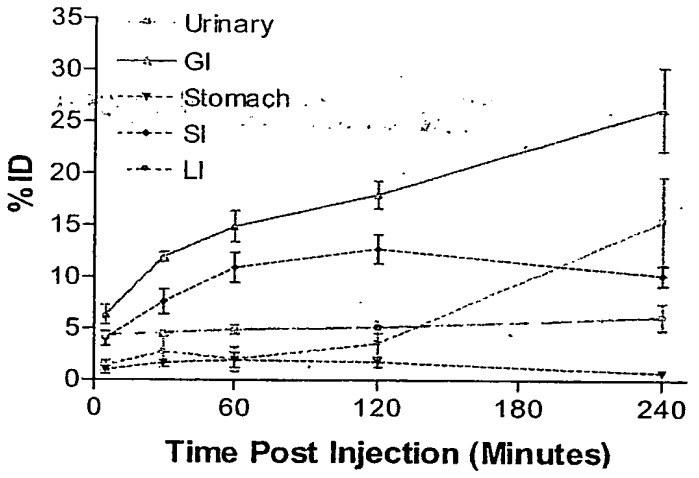

Imaging Agent 2 bound (M)
1.5×10-07
1.0×10-07
5.0×10-08
0.0×10-00
0.0×10-00
2.0×10-05
4.0×10-05
6.0×10-05
8.0×10-05
Imaging Agent 2 added (M)

Figure 7.


30
20
10
0
% ID/G
Blood
Muscle
Tumour
0
60
120
180
240
Time Post Injection (Minutes)

A


35
30
25
20
15
10
5
0
% ID
Urinary
GI
Stomach
SI
LI
0
60
120
180
240
Time Post Injection (Minutes)

B

Figure 8

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INTERNATIONAL SEARCH REPORT

PCT/GB 03/05319

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/18 A61K51/04 A61P9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 02/067761 A (BRISTOL MYERS SQUIBB PHARMA CO) 6 September 2002 (2002-09-06) claims 1,13	1-34
Y	WO 00/78145 A (GAITANOPOULOS DIMITRI E ;SMITHKLINE BEECHAM CORP (US); FRANZ ROBER) 28 December 2000 (2000-12-28) claims 4,5 page 2, line 26 - line 31	1-34

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 April 2004	27/04/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Beranová, P

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 03/05319

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1 - 34 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/GB 03/05319

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